Effects of Temperature Shock on the Hematological Indices of Blackchin Tilapia *Sarotherodon melanotheron* (Rüppel, 1853) Reared in Seawater

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Abstract: ***Farming of tilapia in estuarine is usually accompanied by a fluctuating temperature. Thus, mix sexes of blackchin tilapia S. melanotheron [11.86 ± 1.47 cm TL & 28.75 ± 9.65 g W.] were subjected to three different levels of temperatures [TI(28°C), TII(32°C) and TIII(35°C)] for 24 hrs in seawater condition. Their physiological responses were determined through the following indices in blood: glucose, cholesterol, hemoglobin, hematocrit, red blood cells (RBCs) count, white blood cells (WBCs) count, platelet count, total plasma protein (TPP), serum glutamic pyruvic transaminase (SGPT), and serum glutamic oxaloacetic transaminase (SGOT). Results showed that cholesterol, hemoglobin, hematocrit, red blood cells, white bloods cells, total plasma protein (TPP), serum glutamic pyruvic transaminase (SGPT), and serum glutamic oxaloacetic transaminase (SGOT) were not affected by increasing temperature except blood glucose (p<0.05). These suggest that glucose utilization is a coping mechanism of blackchin tilapia in adapting seawater condition with a fluctuating temperature.***

Keywords: hematocrit, platelet, glucose, cholesterol, serum

1. Introduction

Tilapia is of great importance to the national food security and nutrition, which is second to milkfish fish species cultured in the Philippines. However, tilapia farmers have suffered significant production losses due to extreme climate events such as long heat waves. As a result, tilapia pond culture decreased by 35 percent in the past 8 years, imposing considerable threat to the country's food sufficiency and livelihoods [1]. One of the reasons why tilapia farming is affected is because of the physiological aspect of the fish as reviewed by [2] that suboptimal temperatures have a negative impact on fish immunity and health. This was very clear that water temperature has a pronounced effect on chemical and biological processes in cultured aquatic organisms. Because, a sudden change in temperature of as little as 3°C or 4°C will stress or even kill aquatic organisms [3]. This phenomenon will become worse in the incoming years as global climate change continuously persists [4]. The long-dry season in the Philippines is coupled with its archipelago outline where most of the islands are dependent on the rain water as their freshwater source. However, this setting is predominantly estuarine environments, where shortage of water supply for culture is not an issue because of the seawater. Thus, farming of tilapia in brackish and marine estuarine areas was promoted by the Philippines National Government as the last resort to combat climate change in attaining food security [5]. This goal leads to a selection of a potential species of tilapia that can thrive in both brackish and high saline culture environments. And one of the species that has been eyed is the blackchin tilapia *Sarotherodon melanotheron* (Rüppel, 1853) . This species is known for its wide salinity tolerance, and it has been reported to occur in pure seawater [6]. In their natural distribution, it lives in brackish estuaries and lagoons, rarely in neighboring fresh or saltwater, from Senegal to Zaire [7]. In the Philippines, it is locally called "tilapiang Gloria" referring to the black blotches located on the lower part of the head as the basis of naming by the farmers [6]. It has been reported to occur in Bulacan and Bataan since 2011. And has been harvested and sold in the market. In Iloilo, it is cultured in some rendezvous establishment like in "Pamunit" in Oton that is served after the customer personally caught it with a hook and line as part of their selling strategy. However, the condition of this species in Philippine waters is not yet well studied. Thus, this experiment was conducted to determine the blood parameters of blackchin tilapia *Sarotherodon melanotheron* as an index for their well-being and total health as they existed in an estuarine environment with fluctuating water temperature. This study specifically investigated the concentrations of the following physiological indices in blood such as; glucose, cholesterol, hemoglobin, hematocrit, red blood cells (RBCs) count, white blood cells (WBCs), platelet count, total plasma protein (TPP), serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT).

2. Materials and Methods

2.1 Study site and duration

The study lasted for 24 hrs and was conducted on May 6, 2018 to May 7, 2018 in the Multi-species Hatchery, Institute of Aquaculture (IA), College of Fisheries and Ocean Sciences (CFOS), University of the Philippines Visayas (UPV), Miagao, Iloilo, Philippines (10°38'20.1"N & 122°13"37.0"E).

2.2 Source of experimental organism

One hundred pieces of blackchin tilapia *S. melanotheron* were taken from the settlement pond using cast nets. This pond is located within the hatchery complex about a few meters away from the experimental set-up.

2.3 Experimental organism

Mixed sexes of blackchin tilapia *S. melanotheron* Rüppel, 1853 with a total length and weight (11.86 ± 1.47 cm TL and 28.75±9.65 g W) respectively, were used in the study. Species was classified based on the description of [7] that species is characterized with melanic areas in adult usually present on lower parts of head, on the cleithrum and on apices of caudal and soft dorsal fins, the caudal marks extending along the dorsal and ventral edges of the fin; no vertical bars on the flanks in adults, but occasional irregular and asymmetrical spots probably representing such bars; a median spot or transverse bar on the nape rather constant.

2.4 Acclimation

The test organisms were acclimated in a 1-ton capacity (1pc/10L) circular fiberglass tank for more than a week. It was fed once a day to apparent satiation using a commercial diet. A 50 percent of water level was changed every day to maintain water quality parameters and this was usually done after feeding. Temperature and salinity were measured during acclimation.

2.5 Stocking

Feeding was stopped 24 h before stocking to reduce waste in the experimental tank and ease stress during handling. A 54L capacity rectangular mega box plastic container was filled with 40 liters seawater (35 ppt) and this was stocked with 9 individuals of blackchin tilapia.

2.6 Experimental set-up

The experiment was conducted inside the IA Multi-species hatchery facility. There were three treatments used in the study such as; TI (Control); TII (32^{o}C); TIII (35^{o}C) and this was in a triplicated group with a total of 9 experimental units. Experimental containers were laid out using completely randomized design (CRD).

2.7 Condition of experimental container

Experimental containers were installed with aquarium heaters (Aqua Zonic®) such as 50w in 32^{o}C and 100w for 35^{o}C while for ambient containers there were none. These heaters were installed 24 hours prior to the experiment proper to attain the desired level of temperature in each experimental unit. These containers were not provided with aeration to maintain the temperature and the organisms were not fed to avoid the influence of the diet on the blood chemistry.

2.8 Sampling

Blood sampling was done within 24 h. There were 2 pieces of blackchin tilapia *S. melanotheron* scooped from each container. These organisms were placed in a pail with ice (6^{o}C) for less than 2 minutes to avoid struggling during blood extraction. Each organism was wiped with a clean white mini towel and measured for its weight and total length.

2.9 Blood glucose and cholesterol determination

Blood glucose and blood cholesterol were determined in-situ using glucometer (Accu-Chek® Active, Roche Diabetes Care GmbH, 68305 Mannheim, Germany) and cholesterol meter (EasySure® GCU, Bioptik Technology, Inc., Miaoli County, Taiwan), respectively. After blood withdrawal, caudal peduncle was severed to obtain a drop of blood by squeezing into the glucometer strip and then another drop blood for cholesterol was also obtained through trimming of caudal peduncle until blood oozed out and then slightly touched into cholesterol strip and reading was obtained after a machine counted down for 100 seconds.

2.10 Hemoglobin, hematocrit, red blood cells (RBCs) count, white blood cells (WBCs) count, platelet count, and total protein plasma (TPP) analyses

A 0.5 ml blood was withdrawn through caudal vein puncture using a 1ml syringe with 25 G x 5/8” needle (Cardinal Care ®) which was done at least within three minutes to avoid clotting. This was immediately transferred to 0.5 ml EDTA.K2 capillary blood collection tubes (Guangzhou Improve Medical Instruments Co., Ltd.) and placed in an ice box for transport. These blood samples were brought to the Real Medical Laboratory Diagnostic Center for analysis. Samples were loaded into a machine (Beckman Coulter Act diff) to read the concentration level in test organisms and the results were obtained instantly.

2.11 Serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetate transaminase (SGOT) quantification

Half of the blood volume drawn from the fish caudal was transferred to 1.5ml microcentrifuge tube (Eppendorf®). These tubes were centrifuged at 4^{o}C in low temperature microcentrifuge (HETTICH 4903-02-0) for 10 minutes at 3,000 rpm to separate the serum component of the blood. These serum samples were brought to the Real Medical Laboratory Diagnostic Center within the town for analyses. SGPT and SGOT levels in fish were obtained immediately using the ILAB 300 Plus machine.

2.12 Statistical analysis

Data gathered from the experiment were analyzed using Levene’s test for homogeneity of variances. Homogenized data were run in a one-way analysis of variance (one-way ANOVA) using Statistical Package for Social Sciences (SPSS version 23). Treatments with statistical significance were subjected to Duncan’s Multiple Range Test (DMRT). The level of significance was set at 0.05 and data were presented in mean ± SE in tables.

3. Results

The study was conducted for 24 hrs to determine the overall physiological characteristics of blackchin tilapia *S. melanotheron* in seawater by analyzing its hematological indices after temperature shock. Results showed that there was no significant difference (p>0.05) in blood cholesterol, hemoglobin, hematocrit, red blood cells count (RBC), white blood cells (WBCs), platelet count, total plasma protein (TPP), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) except blood glucose (p<0.05) (Table 1, 2, & 3).

Table 1: Effects of temperature shock on blood glucose, cholesterol, and hemoglobin after 24 hrs of rearing.

Treat.	*Glucose (mg/dL)*	*Cholesterol (mg/dL)*	*Hemoglobin (g/dL)*
28°C	49.00 ± 7.55[b]	405.00±0.00[a]	58.00±10.00[a]
32°C	46.67± 9.07[b]	405.00±0.00[a]	55.33 ±5.13[a]
35°C	75.67±5.69[a]	372.00 ±57.16[a]	56.00 ±19.08[a]

Means having the same letter are not significant (p>0.05).

Table 2: Effects of temperature shock on hematocrit, red blood cells (RBCs) count, white blood cells (WBCs) count, and platelet count after 24 hrs of rearing.

Treat.	*Hematocrit (l/L)*	*RBCs ($x10^{12}/L$)*	*WBCs ($x10^9/L$)*	*Platelet ($x10^9/L$)*
28°C	0.17±0.03[a]	1.66±0.56[a]	3.43±0.46[a]	57.67±6.51[a]
32°C	0.16±0.02[a]	1.88±0.17[a]	3.37±0.32[a]	60.33±6.43[a]
35°C	0.16±0.06[a]	1.68±0.84[a]	3.03±0.61[a]	61.00±11.14[a]

Means having the same letter are not significant (p>0.05).

Table 3: Effects of temperature shock on total plasma protein (TPP), serum glutamic oxaloacetic transaminase (SGOT), and serum glutamic pyruvic transaminase (SGPT) after 24 hrs of rearing.

Treat.	*TPP (g/dL)*	*SGOT (U/L)*	*SGPT (U/L)*
28°C	4.53±2.12[a]	11.00±3.00[a]	20.00±12.12[a]
32°C	4.17±0.95[a]	29.00±26.06[a]	6.33±1.15[a]
35°C	3.13±1.14[a]	26.33±17.93[a]	13.33±10.07[a]

Means having the same letter are not significant (p>0.05)

4. Discussion

The blood glucose is considered as a secondary physiological response of fish as it manifested in hours or even days after the stress occurred [8][9]. In this case, thermal shock was perceived immediately, however, it was reflected in the blood after 24 hours of thermal shock. This indicated that organisms reared in high water temperatures underwent stress, as reported that blood glucose is an indicator of stress in aquatic organisms [10]. Blood glucose in an indicative of retarded metabolism and index of sublethal stress [11].

Blackchin tilapia showed no significant difference in blood cholesterol after 24h of temperature shock. It was assumed that water temperature could modify the rate of lipolysis (mobilization of fat stored). But in this study, it is strongly believed that the temperature tested has no effect on the stored lipid of the species, which in short, it means that the organism didn't undergo stress. And cholesterol might not be a sensitive index for stress in tilapia species. Because of high tolerance to temperature and probably adapted to 35°C, there were no disturbances in the cholesterol level of the species. This contradicts with other findings, where a decrease of fatty acid level was observed and this probably due to an increase in metabolic rate with fatty acid consumption by the tissue [12]. Another study, revealed higher cholesterol, reflected higher energy demand by consumption of fat stored [13].

The figure shows that blackchin tilapia *S. melanotheron* didn't undergo disturbances in respiratory pigment which means that oxygen consumption didn't vary significantly among water temperature levels. The same result was also observed in other fish species, indicating tolerance to the range of temperature variation [12]. Similar result was also obtained in catfish *C. gariepinus* which suggested a high adaptive ability of the species to 35+1°C [11]. No changes in blood hemoglobin was also recorded in red spotted grouper that means species adapts better to 20 and 25°C [14]. This opposed to the work of [15] where decreased hemoglobin was observed, in which modified oxygen quantity that led to slow down metabolic ratio and smaller production energy was observed.

No significant difference was observed in hematocrit among water temperature levels after 24 hrs shock. Hematocrit is a measurement of the cellular fraction of blood and is a common stress indicator for fish [13]. Which means that the blackchin tilapia used in the experiment did not undergo stress for 24 h of exposure to temperature shock. The same result was also reported by another author indicating high adaptive ability of the species [11]. Hematocrit level was not altered by different temperatures, suggesting good tolerance of silver carp [12]. Better adaptation to temperature and no need for extra oxygen was annotated by [14] and [13], respectively, as they obtained no significant changes in hematocrit level of their test animals. In contrast, temperature acts as a stressor in common carp by changing its hematocrit level during the experiment [16].

There was no significant difference (p>0.05) observed in RBCs count of blackchin tilapia after 24 h of the experiment. Opposite findings with other species such as carp reared at 32°C [16] and *O. mossambicus* [17] were reported. The changes observed in those species was a stimulation of erythropoietin for oxygen (O_2) or carbon dioxide (CO_2) transport in fish because of increased metabolic activity [17]. In which this species was not observed, that means blackchin tilapia was totally adapted to existing water temperature.

After 24 h of temperature shock, blackchin tilapia showed no statistical variation in WBC count. This suggests that temperature shock had no effect on the white blood cells count of the species as a stress index. Unlike in common carp that increased in WBC level reared at 32°C, which is an indicator of health. While a decrease in WBC was also reported in *O. mossambicus* in higher temperature suggesting under stress [17].

At the end of the experiment, platelet count revealed no statistical difference in all organisms (p>0.05). Platelets is an autonomous drone for hemostatic and immune surveillance in organisms [18]. These express receptors and a multitude of mediators recognized as being active in inflammation [19]. Platelet functions of adhesion, aggregation and secretion [20]. In this study, it was presumed organisms stocked in different water temperatures will give a different level platelet count as it is an indicator of physiological health of the species. Unlike in humans, platelet count has not been included as indicative of stress or health in fish. This study also suggests that platelet is not a sensitive index for a total health of the organisms. On the other hand, it could also be pointing to the same suggestion that this organism was not affected by the temperature shock. Adaptive strategy of tilapia species was also reported in another study [21].

The figure shows the decreasing concentration of plasma total protein towards higher temperature level persisted throughout the experiment. However, because of wide variation in data distribution it gives no statistical difference (p>0.05). As reported in other species such as in Atlantic halibut, change in total protein is an indication of thermal stress [22]. No significant difference in total protein was also observed in red hybrid tilapia suggesting a coping mechanism of the organism [23]. Disturbance in total protein could lead to growth retardation if continuously persisted. However, as previously reported in *Sarotherodon* sp. reared in 35^{o}C showed no effect on the weight and length relationship, which is a long-term indication of stress.

Serum glutamic pyruvic transaminase activity was not affected by temperature shock in this experiment (p>0.05). This enzyme plays an important role in catabolic and anabolic processes that provide energy in fish. This is called alanine transaminase (ALT) and alanine is a preferred carrier of nitrogen for inter-organ amino acid catabolism [24]. Several amino acids can be converted to alanine, released to the blood, and used as a fuel source in other tissue [25]. This enzyme also provides an intermediate for gluconeogenesis. This could also be used as an indicator of liver cytolysis as it reflects the health of the liver, which is a vital organ in all organisms [26].

The figure above shows that a low level of SGOT activity was observed at ambient temperature but statistically they are not significantly different (p>0.05). This means that the cause of these SGOT levels cannot be attributed to the temperature shock. This could also imply that better adaptation to these temperatures was also the same with other findings [14]. Serum glutamic oxaloacetate transaminase (SGOT) also known as aspartate transaminase (AST) is one of the major glucogenic precursors and important substrate for fish [24]. This enzyme could also be indicating cell damage in the liver, heart, gills, kidney and muscles allowing enzyme to leak out of the cells [26].

5. Conclusion and Recommendation

Blackchin tilapia was highly adapted to the existing water temperature showing their ability to tolerate a wide range of water temperature in hyperosmotic condition. Thus, this means that this species is a potential candidate for brackishwater tilapia farming that could combat fluctuation of water temperature in an estuarine culture environment.

To do a temperature related study, social stress in an aggressive species such as tilapia must be considered. Stock only one individual in a small container to ensure a lone stressor in the culture system. Consider the 10^{o}C range from the ambient temperature for eurythermic species. Therefore, further study on the hormonal effect of temperature shock in tilapia species must be done for a more sensitive index.

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